British Journal of Cancer (1996) 74, (Suppl. XXVII) S161-S163

Tumour blood flow modification by endothelin-related peptides in the rat HSN fibrosarcoma

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Summary Modification of tissue blood flow and tissue vascular resistance was examined in the female CBH rat, bearing a HSN fibrosarcoma, following bolus intravenous administration of 1 nM kg^{-1} endothelin-1 (ET-1) or 1 nM kg^{-1} sarafotoxin S6c (SX6c), selective agonists for endothelin A (ETA) and B (ETB) receptors respectively. Blood flow was measured 15 min after drug administration by the tissue uptake of ^{125}I-labelled-iodoantipyrine. ET-1 and SX6c produced increases in mean arterial blood pressure (MABP) of 52 mmHg and 42 mmHg respectively. Blood flow to the tumour was unaffected by ET-1 treatment, whereas blood flow to normal tissues was reduced, the exception being the heart and the brain in which flow was increased. In contrast, tumour blood flow following SX6c was significantly increased, whereas blood flow in normal tissues was either unaltered or reduced. Vascular resistance was increased in all tissues and the tumour by ET-1 demonstrating that the tumour vasculature was constricting via ETA receptor activation. SX6c however, did not modify tumour vascular resistance, whereas it increased vascular resistance in all normal tissues, suggesting that the tumour lacks a functional population of ETB receptors. This discrepancy may provide a means for selectively modifying tumour blood flow.

Keywords: tumour blood flow; endothelin-1; sarafotoxin S6c; endothelin A and B receptors; vascular resistance

Selective tumour blood flow modification has the potential to enhance some modes of cancer therapy (Jirtle, 1988). Endothelin-1 (ET-1), a recently discovered 21 amino acid peptide, is produced by endothelial cells and has been shown to produce profound vasoconstriction and sustained elevations in blood pressure in all species examined (Yanagisawa *et al.*, 1988). Since its discovery, two other structurally-related endogenous peptides have been identified, namely endothelin-2 and endothelin-3 (Inou *et al.*, 1989). Additionally, four other vasoconstrictor peptides possessing structural homology, the sarafotoxins, have been isolated from the venom of the burrowing Asp, *Atractaspis engaddensis* (Takasaki *et al.*, 1988). Two endothelin receptors have been cloned and are associated with the vasculature (Arai *et al.*, 1990; Sakurai *et al.*, 1990). Firstly, endothelin A (ETA) receptors, which predominate on vascular smooth muscle cells (VSMCs), for which ET-1 displays selectivity and when activated afford potent long-lasting vasoconstriction via phosphoinositol hydrolysis and Ca^{2+} mobilisation. Secondly, endothelin B (ETB) receptors which are present on both endothelial cells (ECs) and VSMCs and for which sarafotoxin S6c (SX6c) possesses high selectivity. When activated, ETB receptors on ECs produce vasodilation by nitric oxide and prostacyclin release, whereas those on VSMCs produce vasoconstriction via an undetermined mechanism.

A down-regulation of ET-1 binding sites has been reported in various tumours (Inagaki *et al.*, 1992; Ben-Baruch *et al.*, 1993; Pekonen *et al.*, 1995), however the capacity of endothelin receptors to modify tumour vascular tone *in vivo* is relatively unknown. This study has therefore been performed to examine the effect of the ETA and ETB receptor selective ligands, ET-1 and SX6c respectively, on blood flow and vascular resistance in the HSN fibrosarcoma, and to compare the results with their vascular effects in normal tissues.

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Materials and methods

Tumours

Early generations of the 3-4-benzpyrene-induced rat HSN fibrosarcoma (Currie and Gage, 1973) (provided by the Institute of Cancer Research, Sutton, UK) were used for these experiments. Maintenance of the tumour line was performed by serial subcutaneous transplantation of 1 mm^3 pieces of the tumour into the left flank of 8-10-week-old female CBH/CBi rats. Transplantation was performed up to 10 passages from the frozen stock, upon which a new sample from the frozen stock commenced the next tumour line. Experimental tumours were grown in the same manner, until they reached between 1 and 2 g in weight.

Drugs

Administration of drug to the rats was by bolus intravenous injection of either 1 nM kg^{-1} endothelin-1 (ET-1) or 1 nM kg^{-1} sarafotoxin S6c (SX6c) (both from Sigma Chemical, Poole, UK). Control rats were treated with 0.9% saline by the same administration route.

Analysis of blood flow

Blood flow was measured using the tissue uptake of the readily diffusible blood flow tracer, ^{125}I-labelled-iodoantipyrine (^{125}I-IAP) (Institute of Cancer Research, Sutton, UK), as described in detail elsewhere (Tozer and Shaffi, 1993). Briefly, rats were anaesthetised by i.p. injection of fluanisone (10 mg kg^{-1}) and fentanyl citrate (0.315 mg kg^{-1}) (Hypnorm; Janssen Animal Health, Oxford, UK) and midazolam (5 mg kg^{-1}) (Hypnovel; Roche Products Ltd., Welwyn Garden City, UK). One tail artery and two tail veins were catheterised with 'Portex' polyethylene catheters (external diameter 0.96 mm, internal diameter 0.58 mm) containing heparinised 0.9% saline. Mean arterial blood pressure (MABP) was recorded throughout by means of an arterial line fitted to a physiological pressure transducer (Gould). Five minutes before blood flow measurement, the rats were heparinised [0.1 ml of 1000 U ml^{-1} heparin (CP Pharmaceuticals Ltd)].

Blood flow to tumours and normal tissues was measured 15 min after the administration of the drug or vehicle, by which time the drug-induced hypertension had been stable for at least 5 min. Free-flowing arterial blood was collected during a 30 s infusion of 0.37 MBq (10 μCi) ^{125}I-IAP prepared in 0.9% saline, delivered by a constant flow infusion pump set at a rate of 1.6 ml min^{-1}. At 30 s, the rat was killed by venous injection of Euthatal (0.3 ml) (RMB Animal Health Ltd., Dagenham, UK) and the tumour, contralateral skin, skeletal muscle, brain, heart, kidney, spleen and small intestine were excised. Blood and tissues were weighed and the ^{125}I levels determined using a Wallac Autogamma well counter.

Tissue blood flow was calculated in ml g^{-1} min^{-1} determined by the mathematical analysis described previously (Tozer *et al.*, 1994), and tissue vascular resistance (TVR) was calculated in res. units [(mmHg) (ml g^{-1} min^{-1})$^{-1}$], determined from the equation,

$$TVR = \text{perfusion pressure/blood flow}$$

where the perfusion pressure was taken to be equal to the measured MABP.

Results

Basal MABP in the anaesthetised CBH rat was 81.4±2.1 mmHg (n=27) and vascular resistance in the HSN tumour was calculated to be 214±14 res. units. Vascular responses to ET-1 and SX6c in the CBH rat were predominantly constrictive as expected, resulting in a MABP at the time of the blood flow analysis of 132.8±4.4 mmHg (n=25) and 123.2±2.0 mmHg (n=6) respectively.

Figure 1 shows changes in the blood flow of the tumour and selected normal tissues following treatment with ET-1 and SX6c represented as fractions of tissue blood flow in control rats. Administration of ET-1 (1 nM kg^{-1}), shown in Figure 1*a*, significantly increased the blood flow in the heart and the brain to approximately 120% of the control flow and reduced the blood flow in all other tissues to between 75% of the control flow, as in the kidney, and 23% of the control flow, as in the muscle. The exception to this was the tumour in which no significant change in the blood flow was observed. Paradoxically, SX6c (1 nM kg^{-1}) increased blood flow to the tumour to 176% of the control value, as shown in Figure 1*b*. At this dose, in contrast to ET-1, SX6c had no effect on the blood flow in the brain, kidney, spleen and heart. However, in a similar manner to ET-1, SX6c decreased blood flow to the skin, muscle and small intestine.

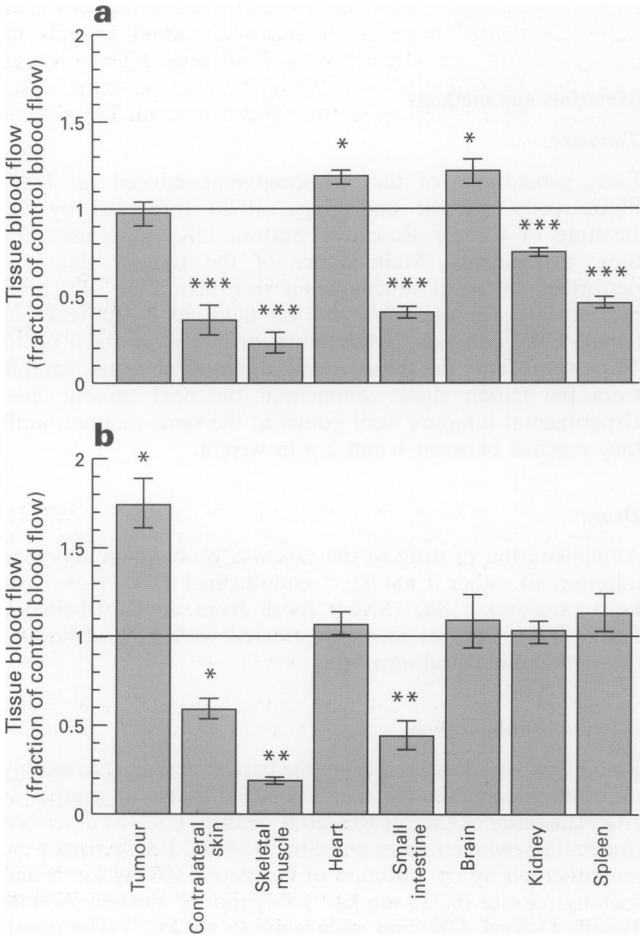

Figure 1 Relative changes in tissue blood flow, 15 min after the administration of (**a**) 1 nM kg^{-1} ET-1 and (*b*) 1 nM kg^{-1} SX6c by bolus intravenous injection in female CBH rats bearing a subcutaneous HSN tumour. Relative changes were calculated as fractions of the control values determined in saline-treated rats. Error bars represent s.e.m. from 25 and 6 experimental observations for ET-1 and SX6c respectively. Significant differences from control values, determined by Student's *t*-test for unpaired data, are indicated. $^{*}P<0.05$, $^{**}P<0.005$, $^{***}P<0.0005$.

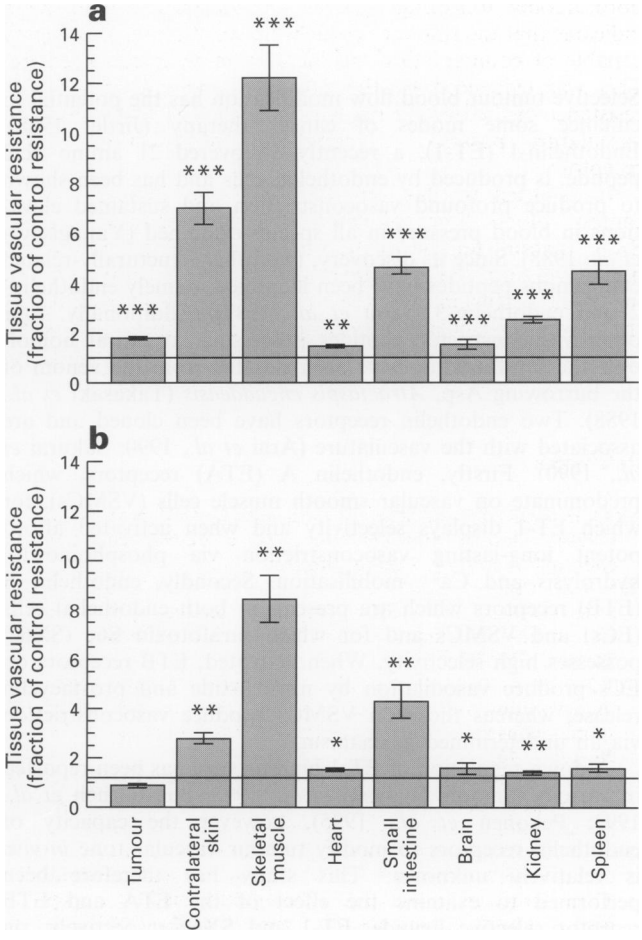

Figure 2 Relative changes in tissue vascular resistance, 15 min after the administration of (**a**) 1 nM kg^{-1} ET-1 and (**b**) 1 nM kg^{-1} SX6c by bolus intravenous injection in female CBH rats bearing a subcutaneous HSN tumour. Relative changes were calculated as fractions of the control values determined in saline-treated rats. Error bars represent s.e.m. from 25 experimental observations for ET-1 and 6 observations for SX6c. Significant differences from control values, determined by Student's *t*-test for unpaired data, are indicated. $^{*}P<0.05$, $^{**}P<0.005$, $^{***}P<0.0005$.

Figure 2 illustrates changes in tissue vascular resistance (TVR), shown as fractions of control vascular resistance following administration of 1 nM kg $^{-1}$ ET-1 (Figure 2*a*) or 1 nM kg^{-1} SX6c Figure 2*b*). Results show significant increases in the vascular resistance of all tissues including the tumour in rats treated with ET-1. Vascular resistance in all normal tissues in SX6c-treated rats increased, but no vasoconstriction was observed in the tumour. The largest response to both peptides was in the skeletal muscle, with ET-1 increasing the vascular resistance to 1200% of that determined in saline-treated rats and SX6c increasing TVR to 840% of the control resistance. Equivalent responses to the equimolar doses of each peptide were observed in most other normal tissues with the exception of the skin in which TVR rose to 700% of the control following ET-1 treatment whereas following administration of SX6c, vascular resistance rose to only 277% of the control value. The other exceptions were firstly the kidney in which 1 nM kg^{-1} ET-1 increased TVR to 250% of the control resistance whereas 1 nM kg^{-1} SX6c only increased the TVR to 140% of the untreated value. The second exception was the spleen in which ET-1 increased TVR to 450% of the control value whereas SX6c increased vascular resistance to only 160% of the resistance determined in saline-treated rats.

Discussion

The lack of modification in blood flow to the HSN fibrosarcoma following systemic administration with ET-1 indicates that the tumour vessels were constricting to a degree capable of counteracting the increase in perfusion pressure. This was evidenced by the calculated increase in vascular resistance of the tumour implying that the HSN fibrosarcoma vasculature possesses a population of functional ETA receptors.

In contrast to this was the significant increase in the tumour blood flow following systemic administration of SX6c, a result unique to the tumour with blood flow to all normal tissues being either unaltered or reduced. The increased perfusion pressure plus the apparent inability of the tumour vasculature to respond as normal tissues by constriction, demonstrated by the lack of vascular resistance modification in the tumour, results in the elevation of the blood flow. This suggests that the HSN tumour vasculature selectively lacks a population of functional ETB receptors, either to induce constriction via VSMC receptors or dilation via EC receptors. This contrasts strongly with all the selected normal tissues in which vascular resistance was significantly increased indicating a predominantly ETB receptor-induced constriction as opposed to dilation in these tissues.

Discrepancies in the degree of constriction produced by ET-1 and SX6c within different vascular beds may be attributed to heterogeneity of the receptor subtype populations, such variations becoming exposed by the receptor subtype selective peptides. However, the slight variation in the resulting perfusion pressure between ET-1- and SX6c-treated rats may also contribute to the variations between the two experimental groups.

Selective differences between endothelin receptor populations in tumours compared with normal tissues have been implicated previously when studies examining ET-1 binding sites observed a far lower level of specific binding in various tumours (Inagaki *et al.*, 1992; Ben-Baruch *et al.*, 1993; Pekonen *et al.*, 1995). This was suggested to relate to a relative lack of smooth muscle cells in tumours, cells which possess a high density of binding sites in normal tissues (Ben-Baruch *et al.*, 1993). These binding studies however did not address vascular receptor functionality or subtypes.

The lack of response of the HSN tumour to the ETB receptor agonist SX6c implies the presence of one functional receptor subtype alone in the tumour vasculature, namely ETA, capable of responding to exogenously administered endothelin-related peptides. If this observation extends to other tumours, the absence of a functional ETB receptor population capable of modifying tumour vascular tone provides a promising means for selective tumour blood flow modification.

Acknowledgements
We would like to thank the Gray Laboratory Cancer Research Trust staff for the care of the animals, and Mrs S Wordsworth for technical assistance. This work was supported by the Cancer Research Campaign.

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